Supplemental Figure Legends

Figure S1. Supplemental data for Figure 1
(A) LLC-PK1 lysates were Western blotted with a monoclonal antibody versus ezrin (green) and the polyclonal antibody versus PDZK1 (red). In the lysate lane to the right, the primary antibodies had been pre-incubated with an excess of soluble PDZK1, while in the lysates lane to the left the antibodies were preincubated with soluble EBP50 (as a control). The PDZK1 signal is lost when the antibody is preincubated with soluble PDZK1, indicating the specificity of the signal. The blots were imaged in two channels with an Odyssey Infrared Imaging System. (B) The indicated cell lines were blotted for EBP50 and E3KARP/NHERF2. Tubulin acts as a loading control.

Figure S2. The ezrin FERM domain increases the affinity of EBP50 for the PDZK1 Tail
(A) In vitro binding assays were performed with the resin-bound GST-PDZK1 tail versus soluble EBP50 and/or ezrin FERM in the indicated combinations. The FERM domain, which runs at the same size as the GST-PDZK1 tail fusion, increases the affinity of EBP50 for the PDZK1 tail. Note that the soluble FERM can be seen in the third pulldown lane due to the increase in signal at the same size as GST-PDZK1 tail. (B) Resin-bound GST-PDZK1 tail was used for precipitations from a constant amount of soluble EBP50 in reactions containing an increasing amount of soluble ezrin FERM domain. Addition of the FERM domain increases the affinity of EBP50 for the PDZK1 tail. The ezrin FERM input lane corresponds to the amount of FERM in the second FERM-containing precipitation.

Figure S3. The GST-PDZK1 tail precipitates EPI64 in a complex with EBP50
In vitro binding assays were performed between resin-bound GST-PDZK1 tail and soluble EBP50 with increasing amounts of EPI64. The EPI64 input lane corresponds to the first pulldown including EPI64 with that amount increased by twofold or fourfold in the following two pulldowns. EPI64 enters into a complex with the PDZK1 tail and EBP50 while mildly inhibiting the association between the PDZK1 tail and EBP50.

Figure S4. Further characterization of Xpress-tagged constructs
(A) The indicated constructs were transfected into JEG3 cells and precipitations were performed with the CNBr resin-bound ezrin FERM domain. The Xpress-Chimera construct is strongly precipitated while there is no evident binding to Xpress-PDZK1 at this exposure. Note that endogenous PDZK1 is precipitated by a similar pulldown in figure 1D. However, in that case there was a much larger endogenous pool to precipitate from, while here the signal from the lysate is far weaker. Together, the results reinforce that some PDZK1 precipitates with the ezrin FERM domain through an EBP50 intermediate, while the PDZK1/EBP50 chimera interacts directly with the ezrin FERM domain. EBP50 is included as a positive control. (B) An image of the apical section of a JEG3 cell expressing Xpress-PDZK1 costained with ezrin. In the merge, ezrin is green while Xpress-PDZK1 is red. The scale bars represent 5 micrometers.

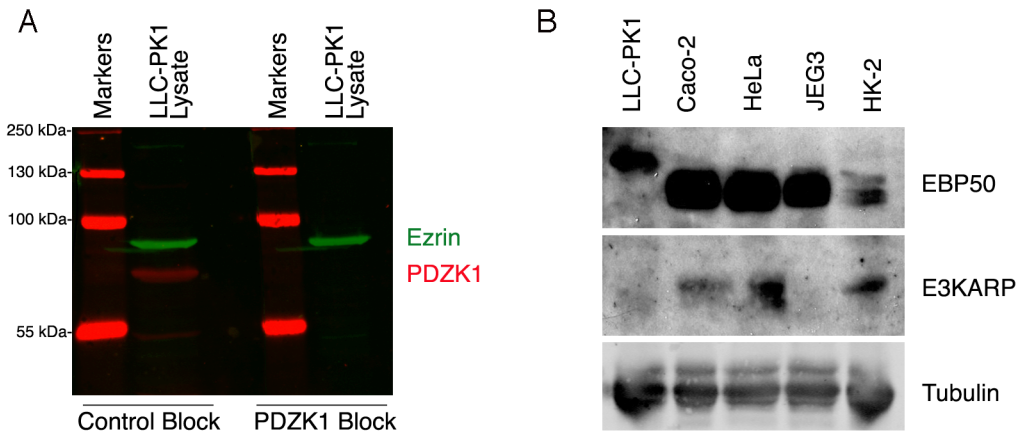
A
Markers
LLC-PK1 Lysate
Markers
LLC-PK1 Lysate
250 kDa
130 kDa
100 kDa
55 kDa
Ezrin
PDZK1
Control Block
PDZK1 Block
B
LLC-PK1
Caco-2
HeLa
JEG3
HK-2
EBP50
E3KARP
Tubulin

Figure S1

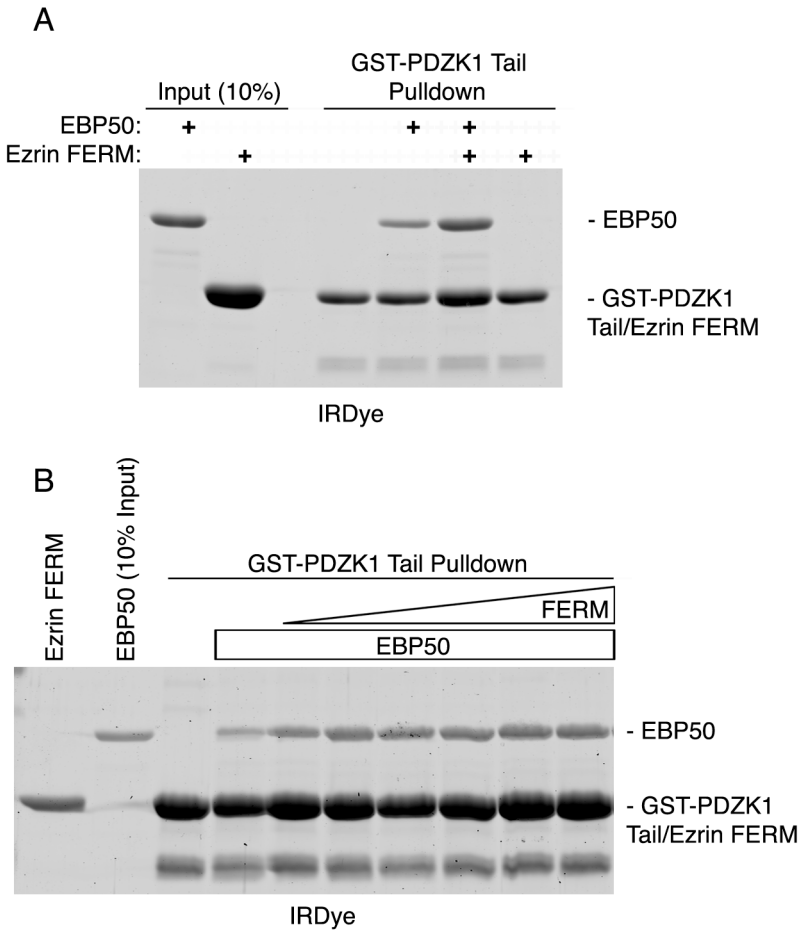
A
Input (10%)
GST-PDZK1 Tail Pulldown
EBP50:
Ezrin FERM:
- EBP50
- GST-PDZK1 Tail/Ezrin FERM
IRDye
B
Ezrin FERM
EBP50 (10% Input)
GST-PDZK1 Tail Pulldown
FERM
EBP50
- EBP50
- GST-PDZK1 Tail/Ezrin FERM
IRDye

Figure S2

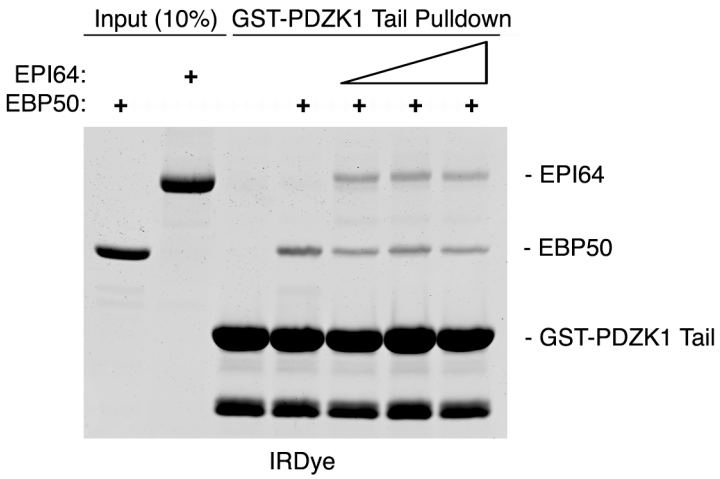
Input (10%)
GST-PDZK1 Tail Pulldown
EPI64:
EBP50:
+
+
+
+
+
+
- EPI64
- EBP50
- GST-PDZK1 Tail
IRDye

Figure S3

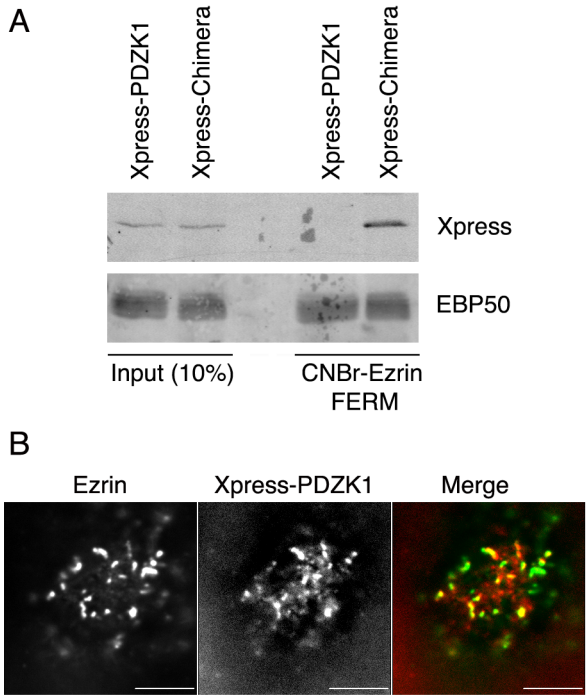
A
Xpress-PDZK1
Xpress-Chimera
Xpress-PDZK1
Xpress-Chimera
Xpress
EBP50
Input (10%)
CNBr-Ezrin FERM
B
Ezrin
Xpress-PDZK1
Merge

Figure S4